

(v) Its melting point is not below 229° C after drying in vacuum at 60° C for 3 hours.

(vi) When calculated on the anhydrous basis, the difference between the absorptivity value at the maximum occurring at 282 nanometers and the absorptivity value at the minimum occurring at 247 nanometers is 100±4 percent of the difference obtained with the gramicidin working standard.

(vii) It is crystalline.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5(b) of this chapter.

(3) *Requests for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, loss on drying, residue on ignition, melting point, identity, and crystallinity.

(ii) Samples required of the batch: Ten packages, each containing approximately 500 milligrams.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.106 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed sample in sufficient alcohol U.S.P. XX to obtain a stock solution of convenient concentration. Further dilute the stock solution volumetrically with alcohol U.S.P. XX to the reference concentration of 0.04 microgram of gramicidin per milliliter (estimated).

(2) [Reserved]

(3) *Loss on drying.* Proceed as directed in § 436.200(b) of this chapter.

(4) *Residue on ignition.* Proceed as directed in § 436.207(a) of this chapter.

(5) *Melting point.* Proceed as directed in § 436.209 of this chapter.

(6) *Identity.* Accurately weigh about 20 milligrams of the sample and dilute in ethyl alcohol to give a concentration of 0.05 milligram (estimated) of gramicidin per milliliter. Prepare a solution of the gramicidin working standard to contain 0.05 milligram per milliliter in ethyl alcohol. Using a suitable recording spectrophotometer with 1-centimeter cells, record the ultraviolet absorbance spectrum of each solution from 220 nanometers to 320 nanometers. The ultraviolet absorbance spectrum of the sample solution

should compare qualitatively to that of the working standard solution. Determine the absorptivities of each at the maximum occurring at 282 nanometers and at the minimum occurring at 247 nanometers (the exact position of the maximum and minimum of the gramicidin working standard should be determined for the particular instrument used).

(7) *Crystallinity.* Proceed as directed in § 436.203(a) of this chapter.

[39 FR 19115, May 30, 1974, as amended at 41 FR 24883, June 21, 1976; 47 FR 23710, June 1, 1982; 50 FR 19920, May 13, 1985]

§ 448.30 Polymyxin B sulfate.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Polymyxin B sulfate is the sulfate salt of a kind of polymyxin or a mixture of two or more such salts. It is a white to buff-colored powder. It is so purified and dried that:

(i) Its potency is not less than 6,000 units of polymyxin B per milligram on an anhydrous basis.

(ii) [Reserved]

(iii) Its loss on drying is not more than 7.0 percent.

(iv) Its pH is an aqueous solution containing 5 milligrams per milliliter is not less than 5.0 and not more than 7.5.

(v) It gives positive color identity tests for polymyxin.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5 of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, loss on drying, pH, and identity.

(ii) Samples required: 10 packages, each containing approximately 300 milligrams.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Add 2.0 milliliters of sterile distilled water to each 5 milligrams of an accurately weighed portion of the sample. Dilute with sufficient 10 percent potassium phosphate buffer, pH 6.0 (solution 6), to give a stock solution containing 10,000 units of polymyxin B

per milliliter (estimated). Further dilute an aliquot of the stock solution with solution 6 to the reference concentration of 10 units of polymyxin B per milliliter (estimated).

(2) [Reserved]

(3) *Loss on drying.* Proceed as directed in § 436.200(b) of this chapter.

(4) *pH.* Proceed as directed in § 436.202 of this chapter, using a solution containing 5 milligrams per milliliter.

(5) *Identity.* (i) To a solution of 2 milligrams of the sample in 5.0 milliliters of water, add 0.5 milliliter of triketohydrindene solution (1:1,000) and 2 drops of pyridine. Boil for 1 minute and cool. A blue color is a positive test.

(ii) To a solution of 2 milligrams of the sample in 5 milliliters of water, add 5 milliliters of sodium hydroxide solution (1:10) and mix well. Add, dropwise, 5 drops of a cupric sulfate solution (1:100), mixing after the addition of each drop. A reddish-violet color is a positive test.

[40 FR 22253, May 22, 1975, as amended at 50 FR 19920, May 13, 1985]

§ 448.30a Sterile polymyxin B sulfate.

(a) *Requirements for certification—(1) Standards of identity, strength, quality, and purity.* Polymyxin B sulfate is the sulfate salt of a kind of polymyxin or a mixture of two or more such salts. It is a white to buff-colored powder. It is so purified and dried that:

(i) Its potency is not less than 6,000 units of polymyxin B per milligram, on an anhydrous basis. If it is packaged for dispensing, its content is satisfactory if it is not less than 90 percent and not more than 120 percent of the number of units of polymyxin B that it is represented to contain.

(ii) It is sterile.

(iii) It is nonpyrogenic.

(iv) [Reserved]

(v) Its loss on drying is not more than 7.0 percent.

(vi) Its pH in an aqueous solution containing 5 milligrams per milliliter is not less than 5.0 and not more than 7.5.

(vii) Its residue on ignition is not more than 5 percent.

(viii) If it is intended for systemic medication, its heavy metals content is not more than 100 parts per million.

(ix) It gives positive color identity tests for polymyxin.

(2) *Labeling.* In addition to the requirements of § 432.5 of this chapter, if the drug is packaged for dispensing its labeling shall bear the statement, "Caution: This drug should be given intramuscularly and/or intrathecally only to hospitalized patients so as to provide constant supervision by a physician".

(3) *Request for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, sterility, pyrogens, loss on drying, pH, residue on ignition, heavy metals, and identity.

(ii) Samples required:

(a) If the batch is packaged for repackaging or for use as an ingredient in the manufacture of another drug:

(1) For all tests except sterility: 10 packages, each containing approximately 300 milligrams.

(2) For sterility testing: 20 packages, each containing approximately 300 milligrams.

(b) If the drug is packaged for dispensing:

(1) For all tests except sterility: A minimum of 10 immediate containers plus one additional package containing 1 gram of the batch.

(2) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay—(1) Potency.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed sample in 2 milliliters of sterile distilled water for each 5 milligrams of weighed sample. Further dilute an aliquot of this solution with sufficient 10 percent potassium phosphate buffer, pH 6.0 (solution 6), to give a stock solution of convenient concentration; also, if it is packaged for dispensing, reconstitute as directed in the labeling. Then using a suitable hypodermic needle and syringe, remove all of the withdrawable contents if it is represented as a single dose container; or if the labeling specifies the amount of potency in a given volume of the resultant preparation, remove an accurately measured representative portion